special report

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Proposed Guidelines for Thyroid Test Utility

The following Special Report from the Committee on Nomenclature of the American Thyroid Association (ATA) is an attempt to clarify some of the confusion that exists on the use and performance of the many various methods for free thyroxin (FT_4) and to update the performance guidelines of the newer "highly sensitive" thyrotropin (TSH) sandwich-type assays. In 1987, the ATA through this Committee published a report on the recommended nomenclature for tests of thyroid hormone assessment. This was followed in 1990 by a second report recommending to clinicians the proper thyroid test algorithm for thyroid disease screening. This third report now clarifies some of the issues and concerns regarding the different options for FT_4 methods and discusses the committee's consensus and guidelines regarding the utility of the newest, so-called third-generation "sensitive" TSH assays.

Timely reports such as these are important contributions that help dispel the confusion that laboratorians have regarding which test approach is best and what are the experts' opinions concerning appropriate clinical utility. This report also exemplifies the cooperative spirit that currently exists between the ATA and the AACC. Three of the current members of the ATA Commission on Nomenclature are AACC members.

It is important that provoking, penetrating consensus reports on specific analytes continue to appear from time to time. As with the consensus document published several years ago on cyclosporine monitoring, these reports are very useful to laboratory directors in their decisions on which tests are most efficient and appropriate for a particular clinical setting and what guidelines should be followed to ensure acceptable test performance. The authors of this special report are to be commended for their effort in clarifying a somewhat muddied topic.

Laurence M. Demers

Department of Pathology
M. S. Hershey Medical Center
Hershey, PA 17033

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American Thyroid Association Assessment of Current Free Thyroid Hormone and Thyrotropin Measurements and Guidelines for Future Clinical Assays

Ian D. Hay,[1] Monika F. Bayer,[2] Michael M. Kaplan,[3] George G. Klee,[4] P. Reed Larsen,[5] and Carole A. Spencer,[6] for the Committee on Nomenclature of the American Thyroid Association[7]

During 1987, the American Thyroid Association (ATA) Committee on Nomenclature issued a report intended to clarify and simplify the current nomenclature for tests of thyroid hormones and thyroid-related proteins in serum.[8] The 1987 report provided guidelines for classification of these tests; a second report, published in 1990, outlined for clinicians a suggested approach for using these tests in the laboratory diagnosis of thyroid disorders. This second report recommended that, at the present time, the principal laboratory tests for thyroid disease should be estimation of free thyroxin (FT_4) and a "sensitive" thyrotropin (TSH) assay. Two issues raised in these reports that clearly needed additional comment were (*a*) the new approaches to free thyroid hormone measurement, and (*b*) the diagnostic utility of the newer, more sensitive TSH immunometric

[1] Division of Endocrinology and Internal Medicine, Mayo Clinic, Rochester, MN 55905.
[2] Division of Nuclear Medicine, Stanford University School of Medicine, Stanford, CA 94305.
[3] 29877 Telegraph Road, Southfield, MI 48076.
[4] Division of Metabolic and Hematologic Biochemistry, Mayo Clinic, Rochester, MN 55905.
[5] Thyroid Diagnostic Center, Brigham and Women's Hospital, Boston, MA 02115.
[6] Department of Medicine, University of Southern California School of Medicine, Los Angeles, CA 90033.
[7] Address reprint requests to the American Thyroid Association, Endocrine–Metabolic Service, Walter Reed Army Medical Center, Washington, DC 20307 (Leonard Wartofsky, M.D., Secretary of the Association).
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[8] Nonstandard abbreviations: ATA, American Thyroid Association; T_4, thyroxin; T_3, triiodothyronine; FT_4, free thyroxin; FT_3, free triiodothyronine; TSH, thyrotropin; FT_4I, free thyroxin index; FT_3I, free triiodothyronine index; IMA, immunometric assay; TBG, thyroxin-binding globulin; THBR, thyroid hormone binding ratio; TBPA, thyroxin-binding prealbumin; FDH, familial dysalbuminemic hyperthyroxinemia; and NTI, nonthyroidal illness.

ıssays (IMAs). The third of these reports now focuses on ırrent methods for FT_4 estimates and TSH IMA, and ffers analytical and performance guidelines that hould be relevant to those laboratories and clinics hich have been advised by the ATA to use such tests in ıe routine investigation of possible thyroid dysfunc-on.

easurement of Free Thyroid Hormones

The very small free concentrations of thyroxin (T_4) ıd triiodothyronine (T_3) in the blood, i.e., the amounts ıt bound to serum proteins, are generally believed to ısely reflect the hormonal states of hyperthyroidism d hypothyroidism. For this reason, the direct mea-rement of FT_4 is theoretically an optimal test of roid function. Unfortunately, however, the direct asurement of serum FT_4 and free T_3 (FT_3) concentra-ns presents a considerable technical challenge for ee principal reasons. First, the free concentrations only about 0.03% of the total serum T_4 and about % of the total serum T_3 concentration, thus requiring ubpicomole sensitivity of the analytical methods. ond, it is important to avoid disturbing the equilib-n between bound and free hormone in the course of measurement. Third, sera from some patients, espe-ly patients with nonthyroidal illnesses, appear to ain inhibitors and other interferences that can in-date these measurements. Although these inhibi- are generally considered to be an in vitro phenom-, they may also play a role in the FT_4 concentration vo so that the measurements in their presence may ed reflect free hormone concentrations.

t Measurement of FT_4 and FT_3

irect" FT_4 and FT_3 assays currently serve mainly eference" techniques. These assays have in the past technically demanding, relatively expensive, time uming, and therefore unsuited for a high-volume al laboratory. These direct FT_4 methods involve l physical separation of the free hormone from the l hormone by equilibrium dialysis or ultrafiltra- and quantification of the analytes by either radio-unoassay or chromatography. Only minimal dilu-of the serum is permissible, because dilution of n sera may distort the equilibrium between bound ee thyroid hormone concentrations by altering the ıg of endogenous anions, drugs, and other compet-of T_4 binding to serum proteins. Some clinical tories are using a simplified equilibrium dialysis d that is now commercially available.

ther available methods of assessing FT_4 and FT_3 trations are "indirect," and are most properly ed as estimates of FT_4 and FT_3. This includes the ıction derived from isotopic equilibrium dialysis sed below), even though this method has func-as the accepted reference method ("gold stan-for more than 20 years. It is notable, and disturb-at the mean serum FT_4 concentration in normal tions, as determined by different direct methods indirect estimates derived from equilibrium dialysis, varies substantially among laboratories. Moreover, it is unfortunate that no serum standards (or serum surrogate preparations) are currently available for independent calibration of methods, even reference methods.

Indirect Methods of FT_4 and FT_3 Estimation

Because the direct reference methods for FT_4 and FT_3 determination are cumbersome, several easier approaches have been developed for FT_4 and FT_3 estimation; most are available commercially in kit form. Three broad categories are in use: index methods, two-step immunoassays, and analog (one-step) immunoassays.

Index methods. The FT_4 index (FT_4I) and FT_3 index (FT_3I) were originally developed to correct the serum total hormone concentrations for abnormalities in thyroxin-binding globulin (TBG), most commonly those induced by pregnancy or estrogen therapy. Index methods involve two separate methodologies: measurement of serum total T_4 or T_3 by immunoassay, and an assessment of either the serum TBG concentration or the free fraction of the hormone. Several isotopic methods have been used to estimate the free hormone fraction: equilibrium dialysis, ultrafiltration, and T_3 or T_4 "uptake" methods. FT_4I and FT_3I values calculated from serum TBG or uptake methods are theoretically proportional to the actual free hormone concentration but not equal to it. Isotopic equilibrium dialysis and ultrafiltration are considered to give accurate measurements of the free hormone fraction, and therefore may allow calculation of absolute FT_4 and FT_3 concentrations. Unfortunately, the reported free fraction of T_4 in normal serum as measured by equilibrium dialysis in different laboratories has varied by as much as twofold. Possible confounding variables include the presence of minor impurities in the tracer, the temperature and (or) duration of dialysis, the constancy of the dialysis, adsorption of tracer by components of the dialysis system, and the possible in vitro generation of free fatty acids.

FT_4I and FT_3I values based on uptake methods involving isotopic or nonisotopically labeled T_4 or T_3 remain in wide use. Their performance is variable, as judged by comparisons with reference equilibrium dialysis methods, but they have the advantages of being simple, rapid, and inexpensive. The values obtained by uptake methods should be expressed as a thyroid hormone binding ratio (THBR), which is directly proportional to the free hormone fraction (within limits) when the calculation is performed correctly, as reviewed in the 1987 ATA Committee on Nomenclature report.

Two-step immunoassays. In the first step, FT_4 (or FT_3) is immunoextracted from serum by an antibody of an appropriate specificity bound to a solid support, often a coated tube. After washing, a second step introduces T_4 (or T_3) tracer, which binds to the remaining unoccupied antibody sites. Thus, the FT_4 (or FT_3) concentration is inversely related to the antibody-bound radioactivity, and a standard curve is generated from secondary standards calibrated by a reference method.

Analog immunoassays. In these methods, the tracer is

^{125}I-labeled T_4 or T_3 analogs of undisclosed chemical composition that, in theory, bind to anti-T_4 or anti-T_3 antibodies but not to serum thyroid hormone binding proteins. Unfortunately, this premise has been difficult to realize in practice, and studies have shown significant binding of the synthetic tracers to serum thyroid hormone binding proteins, especially thyroxin-binding prealbumin (TBPA) and albumin. Thus, there is considerable debate about how accurately the currently available kits estimate serum free hormone concentrations. Despite constant reformulation intended to overcome dependence of free thyroid hormone results on serum albumin and TBG concentrations, these assays still have problems.

Strengths of FT_4 and FT_3 Estimate Methods

The advantages of these methods over the reference methods are wider commercial availability, technical ease, and rapid throughput. They give results diagnostically comparable with those of reference techniques in normal subjects, hyperthyroid and hypothyroid patients, and patients with the common mild abnormalities in serum TBG concentrations consequent to pregnancy or treatment with estrogen. When a method is to be used only in these populations, the specific choice can be reasonably based on such factors as overall analytical accuracy, cost, and technical ease.

Weaknesses of FT_4 and FT_3 Estimate Methods

The disadvantage of the estimate methods is variable diagnostic accuracy in euthyroid patients who show extreme abnormalities in T_3 and T_4 binding to serum proteins. Such conditions are encountered frequently in endocrinology specialty practices and very commonly in hospital inpatient services. Particularly common problems include abnormalities in TBG or TBPA concentrations, familial dysalbuminemia, T_4 or T_3 autoantibodies, and nonthyroidal illness (NTI). These problems are further discussed below.

Congenital TBG excess or deficiency. Prevalence estimates are 3:100 000 for congenital TBG excess and 1:10 000 for congenital TBG deficiency. Patients with these conditions are believed to be euthyroid, and their serum FT_4 and FT_3 concentrations are normal by reference methods. Because TBG binds both T_4 and T_3, total serum concentrations of both iodothyronines will be increased and the THBR will be low in the case of TBG excess. However, the extent of the binding abnormalities in these patients is often beyond the limit of the linear relationship of the THBR with the free fraction of T_4 or T_3. Accordingly, the FT_4I and FT_3I can be abnormal, and in these circumstances, the analog methods may also give abnormal results.

Familial dysalbuminemic hyperthyroxinemia (FDH), TBPA excess, and T_4 or T_3 autoantibodies. FDH is the result of congenital excess of a normally minor component of serum albumin, for which the affinity for T_4 is much higher than that of the bulk of the albumin protein; the affinity for T_3 is unchanged. The patients are clinically euthyroid and, although their total serum T_4 concentrations are increased, their serum FT_4 and FT_3 concentrations are normal by reference methods. The prevalence of this condition is estimated to be about 1:10 000 but may be higher in some populations, e.g., Puerto Ricans. Because T_3 binds to the abnormally increased albumin subfraction with an affinity much less than that of T_4, FT_4I methods based on a THBR that includes T_3 will typically overestimate the FT_4. Likewise, several analog methods of FT_4 estimation give artifactually high values of FT_4 in affected subjects, but in this case these increased values result from the binding of the analog to the variant albumin. The very rare congenital TBPA excess and T_4 autoantibodies, which are occasionally present in autoimmune thyroid diseases, may give results similar to those in FDH with some FT_4I methods.

Nonthyroidal illness. Several abnormalities of circulating thyroid hormones occur in NTI. The most common abnormality is a decrease in serum T_3 concentration. Also common are increases in the free fractions of serum T_4 and T_3, caused by decreases in serum concentrations of the binding proteins, alterations in binding properties induced by endogenous binding inhibitors and drugs, or some combination of these mechanisms. Total serum T_4 concentrations may fall and there is an inverse correlation between serum T_4 concentrations and the severity of the illness. The total serum T_4 concentration may occasionally be increased. Serum FT_4 concentrations in NTI patients with low or high total T_4 values are usually normal or even above-normal by reference techniques. Clinical evidence is lacking for a hypothyroid state in patients with severe NTI and subnormal serum T_4 values. Their serum TSH concentrations are usually normal, and thyroid hormone therapy does not improve their prognosis. Nevertheless, the relationships between circulating FT_4 and FT_3 concentrations, intracellular and nuclear T_4 and T_3 concentrations, and the patients' thyroid status at the tissue level remain uncertain.

The practical diagnostic problem is to distinguish patients with severe NTI plus hypothyroidism from those with low serum T_4 due to NTI alone. The prevalence of the low-T_4 state in NTI varies widely according to the patient population, but in the intensive-care setting, NTI is a much more common cause of low T_4 than is primary hypothyroidism. Accurate diagnosis in this setting therefore demands an FT_4 estimate that correctly reflects the serum FT_4 concentration or, alternatively, a heavier reliance on the concentration of serum TSH, as measured in a "sensitive" assay. Because of the multiple mechanisms for abnormalities in serum thyroid hormone concentration and binding during NTI, this common clinical situation poses a difficult challenge to the accurate determination of free serum thyroid hormones and probably results in the greatest number of inaccurate test results.

Comparative Accuracy of FT_4 and FT_3 Estimate Methods

Many of the FT_4 estimate techniques give abnormal values for patients with major quantitative or qualita-

ive changes in T_4 binding to serum proteins. These bnormalities, summarized in Table 1, deviate from the enerally normal values obtained by reference (direct quilibrium dialysis/RIA) techniques, and from the pa-ents' apparently euthyroid clinical status. If these rtifactual abnormalities are not recognized, inappro-riate patient management may result.

FT_3, however measured, is not currently recom-ended as a diagnostic test in NTI because it offers ttle additional information to that provided by deter-ination of total serum T_3 concentrations. Moreover, ie concentrations of either FT_3 or total T_3 reflect more e peripheral de-iodination rates of T_4 rather than the yroidal hormone secretory rates. FT_3I methods usu-ly give normal results in FDH, but little information available about the performance of these assays in the her clinical situations that present difficulties for FT_4 timate techniques.

oblems with Current Free Thyroid Hormone Assays

Most reference methods for FT_4 and FT_3 determina-ns are not practical for use in clinical laboratories, t the choice of an FT_4 estimate method appropriate for clinical situations is at present difficult, for several asons: (*a*) Kit manufacturers frequently fail to provide rformance data in their package inserts for patients th major protein binding abnormalities or with the v-T_4 state of NTI; indeed, package inserts often make mention of these potential inaccuracies. (*b*) There are guidelines for the types of NTI or drug interactions it the manufacturers should evaluate. (*c*) Published nparative studies of different methods rapidly become dated because of the frequent reformulation of the ducts, often with no indication to users. (*d*) The gnostic accuracy of a method cannot always be pre-ed on the basis of the analytical method: e.g., in ients with the low-T_4 state of NTI, most FT_4I kit thods will give low results but some consistently vide normal values.

These practical problems are superimposed on un-avoidable uncertainties dictated by pathophysiology. Because the reference ranges of normal serum FT_4 and FT_3 concentrations, defined by 95% confidence limits, may vary over more than a twofold range, different laboratories may display considerable overlap between the normal range and the values in patients with both thyroid and nonthyroid diseases. Therefore, diagnostic accuracy will depend on the prevalence rates of the various abnormalities in the patient population being served, as well as on analytical accuracy. These prevalence rates are often unknown and hardly ever controllable.

Guidelines for Future Free Thyroid Hormone Assays

Although equilibrium dialysis (or ultrafiltration) of minimally diluted sera followed by RIA of the dialysate (or ultrafiltrate) for T_4/T_3 is generally recognized as an important reference method of measuring free thyroid hormones, the availability of such a method has in the past been limited and methodological standardization has been poor. Few investigators have appropriate antisera, so the procedure is therefore not widely available. The production of high-affinity antisera for T_4 and T_3 and the development of a suitable reference method, available in kit form, should therefore become high priorities with manufacturers.

Reference methods for "direct" assays should conform to fairly stringent performance characteristics so that kit manufacturers, investigators, and clinical laboratories would be inclined to accept and use them for evaluation and validation of newer FT_4 estimate methods. Because the vast majority of assays in clinical use are developed commercially, it would be in the best interest of high-quality clinical service if manufacturers would concentrate more on the analytical and diagnostic accuracy of their assays, rather than the ease of use and costs. We believe that manufacturers of FT_4 and FT_3 kits should be required to provide Scatchard plot analyses indicating that the antisera in the kits (*a*) have sufficiently high affinity constants to be capable of

Table 1. FT_4 Measurements in Common Conditions Affecting Thyroid-Binding Proteins

Clinical condition	"Direct" equilibrium dialysis/RIA[a]	Other index methods	Two-step methods	Analog methods
r normal concn of serum binding proteins				
ɔthyroidism	L	L	L	L
ɜrthyroidism	H	H	H	H
ɜrestrogenism	N	N	N	N
ɔrmal concn of serum binding proteins				
excess	N	H	N	L, N, or H
deficiency	N	L	N	L, N, or H
ɹlbuminemia	N	N–H[b]	N	H
ɜalbuminemia	N	N	N	N–L
ɹtoantibody	N	H	N	H
T_4 NTI	N–H	L	N–L	L
T_4 NTI	N–H	N–H	N–H	H

- low; H = high; N = normal.
ethods involving minimal dilution.
ɔrmal when determined with labeled T_4, high with labeled T_3.

measuring ambient FT_4 concentrations and (*b*) are used in sufficiently low concentrations to ensure that, unless there is physical separation of the free hormone before analysis, <1–2% of the total T_4 present is bound, thus avoiding any significant perturbation of the equilibrium between bound and free hormone.

When a new FT_4 estimate method is proposed, this ATA Committee recommends that the manufacturer or investigator should have evaluated the following characteristics and should state their findings in assay descriptions:

- The composition of all assay reagents and components should be disclosed and the characteristics of the antisera used should be provided.
- For "analog" methods, the chemical structure of the analog and adequate data on its binding characteristics with albumin, TBG, and TBPA, without and with "blockers," should also be routinely provided.
- Manufacturers or investigators should be encouraged to clarify the theoretical principles on which their proposed new tests are based. For "analog" assays, evidence also ought to be provided to demonstrate that the binding of the tracer analog to albumin, TBG, or TBPA is minimal. Otherwise, measurements with such an assay would be unlikely to truly reflect the free hormone concentrations.

Performance data on new FT_4 estimate methods should also include

- Data on precision and reproducibility, especially at the critical decision limits (upper and lower limits of normal range).
- An assessment of linearity, based on known additions of T_4 and TBG or T_4 and other binders.
- A definition of the normal range in ambulatory patients.
- An assessment of cross-reactivity with chemically related substances, e.g., other iodothyronines.
- Data on interfering substances and drugs, as they become available.
- Data on analytical accuracy obtained primarily by comparative evaluation with the reference method, not only for sera from ambulatory patients with thyroid dysfunction but also for sera from patients with binding protein abnormalities, sera from patients with the low-T_4 state of NTI, and sera to which free fatty acids or drugs have been added.
- It would also be desirable for manufacturers to demonstrate that their FT_4 estimates measured in critically ill patients are clearly distinguishable from those of hypothyroid patients with comparably low concentrations of total T_4.

Thyrotropin Measurements

TSH is a glycoprotein secreted by the anterior pituitary in response to stimulation by thyroliberin (thyrotropin-releasing hormone) from the hypothalamus; it is inhibited by T_4 and T_3 derived from the thyroid gland. TSH is composed of two subunits, the alpha subunit being common to other glycoprotein hormones (follitropin, lutropin, and choriogonadotropin). Circulating concentrations of TSH are similar for both sexes, with higher values being found in the first few weeks of life. A small but significant circadian rhythm, with peak values occurring in the late evening and near midnight, does not influence the clinical utility of the measurement.

Historically, serum TSH measurements by RIA were used to assess primary hypothyroidism, which is associated with increased TSH. The introduction of more sensitive IMA methods expanded the clinical role of TSH measurements to include the assessment of thyrotoxicosis, a situation associated with subnormal circulating concentrations of TSH. Whereas all immunoassay methods can readily detect increases in serum TSH, only the newer IMAs can detect suppressed concentrations within time frames and with measurement protocols practical for clinical laboratories.

Principle of Immunometric Assays of TSH

These newer TSH IMA or "sandwich" systems make use of two or more antibodies or antisera directed to different portions of the TSH molecule, such that the analyte is "sandwiched" between the antibodies. One of the two antibodies is attached to a solid-phase separation system and extracts the TSH molecules from a serum sample. The second anti-TSH antibody has a signal (radioisotopic, enzyme, fluorophor, or chemiluminescent molecule) associated with it, enabling one to quantify the antibody-bound TSH. The TSH molecules form a "bridge" between the two antibodies, and the measured signal increases in direct proportion to the increasing TSH concentration.

The multiple signal and separation systems developed have given rise to a new lexicon of assay names. When a radioactive tag such as ^{125}I is used, the assay is called an immunoradiometric assay (IRMA). When enzymes such as peroxidase or alkaline phosphatase are used, the assay is called an immunoenzymometric assay (IEMA). When chemiluminescent compounds such as luminol, dioxetanes, or acrydinium esters are used for the signal, the result is an immunochemiluminometric assay (ICMA).

The separation systems used do not alter the assay names but do influence the procedure and equipment needed for the assay. In some assays, the antibody for separation is immobilized on test tubes or plastic beads, so that bound and free analyte are separated by decanting and washing. In others, this antibody is attached to ferromagnetic particles and separated by magnets. Another variation is to attach biotin (a high-affinity binder of avidin) to the antibody and separate the TSH sandwich complexes by avidin linked to a solid phase. In other TSH IMA systems, glass fiber paper has been used to adsorb the anti-TSH antibody.

In contrast to RIAs, where a limited amount of antibody binds native and labeled analyte competitively, these IMA systems use much larger quantities of antisera because an excess of the first antisera is needed to bind the majority of the analyte present in a serum sample. This creates a positive dose–response curve; i.e.,

the signal increases with analyte concentration, in contrast to the inverse dose–response curves found in RIA.

Evaluation of Analytical Sensitivity

The phrase "analytical sensitivity" (detection limit) has been used in multiple ways to describe the lowest concentration that can be reliably measured. Traditionally, this lower limit has been defined as the concentration corresponding to the mean plus or minus 2.5 times the standard deviation of signal response for the zero calibrator assessed in a within-assay mode. This is the concentration that gives an average response statistically different from the noise limits of the signal produced by a specimen with zero concentration of the analyte. However, for a specimen that contains exactly the threshold concentration of analyte, one-half of the times that such a specimen is measured, the signal produced will be below the stated sensitivity limit. Therefore, this is not truly a concentration that can be measured, unless replicate measurements are performed in the same assay and averaged, which is not the case in which clinical specimens are normally analyzed. Also, it may be misleading to compare the absolute values for analytical sensitivities of different assays, defined on the basis of the noise of the zero calibrator, if the various assays use different calibrators, constructed from different non-human-based serum matrixes, which may react differently from patients' specimens that contain zero TSH concentration.

An alternative method for evaluating analytical sensitivity is to determine the adequacy of the TSH assay in discriminating TSH concentrations in sera from euthyroid subjects from those obtained from healthy hyperthyroid subjects. The ATA Committee on Nomenclature previously proposed that sera from hyperthyroid subjects should give results that are more than 3 logarithmic SD below the mean value found in sera from normal subjects. This criterion is based on the assumption that the distribution of the logarithm of TSH values in ambulatory healthy subjects is approximately gaussian so that <0.135% of healthy subjects would have values below the mean minus 3 geometric SDs. This criterion would assure good separation if the measured values of the sera from hyperthyroid subjects were very low and tightly distributed around their mean. Unfortunately, most assays show considerable variation in the low range. However, the precision could be statistically improved by measuring replicates and averaging the results, although this might cause problems in assay turnaround. The variation of the measurement at low concentration can be determined analytically by making pools from mixtures of the zero serum calibrator and the next calibrator and performing repetitive measurements of these pools. These precision profile data then can be used to calculate the overlap between measurements from clinically hyperthyroid and euthyroid subjects. For clinical decisions, the lower the overlap the better, but at least the overlap should be <5%.

Analytical and Performance Guidelines for Future Serum TSH Assays

This ATA Committee recommends that the following characteristics be evaluated by manufacturers and the findings stated explicitly in package inserts:

- The functional detection limit of all future serum TSH assays should be clearly defined on the basis of interassay precision characteristics, this being the mode used in clinical laboratory practice.
- Percent cross-reactivity with lutropin, follitropin, and human choriogonadotropin should be calculated as 10 times the inverse of the concentration of cross-reactant that gives a signal equal to signal obtained with 10 milli-int. units of TSH per liter. The cross-reactivities with each of these glycoprotein hormones should be <0.1%.
- Linearity of response: results for patients' specimens diluted in the manufacturer's indicated diluent (the composition of which should be stated) should be parallel to the standard curve within ±10%.
- Recovery: standard material added to patients' specimens should be recovered within ±10% over the entire range of the assay.
- Accuracy: the average assay results for WHO or MRC Standards, when added to the assay diluent, should agree within ±5% of the stated value.
- Precision: the interassay CV should be optimally 10–15% and preferably <20% at a serum TSH concentration of 0.1 milli-int. unit/L.
- Matrix effects: sera from thyrotoxic individuals should read within ±5% of the signal for the assay zero calibrator.
- Interference: assays should be free from significant interference from heterophilic antibodies. Mouse sera or IgG preparations (e.g.) should be added to eliminate this problem and their concentrations stated.
- "Hook" effect: the standard curve should not flatten or turn down in the presence of TSH concentrations ≤300 milli-int. units/L, e.g., in congenital hypothyroidism.
- In reporting a decision threshold for separating TSH values from hyperthyroid subjects from euthyroid subjects, the criteria for selecting subjects and the number of subjects tested in each group should be stated, along with the percentages above or below the decision threshold. The overlap of these groups should be <5%, preferably <1%.

It is our recommendation that, for a newer assay method to be classified as a truly "sensitive" TSH assay, having the potential for use as a first-line test of thyroid function, it should conform to these proposed analytical and performance guidelines. At present, the majority of commercially available TSH IMAs appear capable of distinguishing normal from hyperthyroid values in ambulatory patients but cannot reliably differentiate between the mildly subnormal values (0.01–0.1 milli-int. unit/L) seen in hospitalized or T_4-treated patients and the profoundly low values (<0.01 milli-int. unit/L) typical of thyrotoxicosis. It is our expectation that newer TSH assays will show further improvement in analyti-

cal sensitivity and that such improvements will permit an easier distinction between mildly subnormal TSH concentrations (such as may be seen with NTI or corticosteroid treatment) and frankly hyperthyroid values, as well as allow the quantification of degrees of thyrotropin suppression in subclinical hyperthyroidism.

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